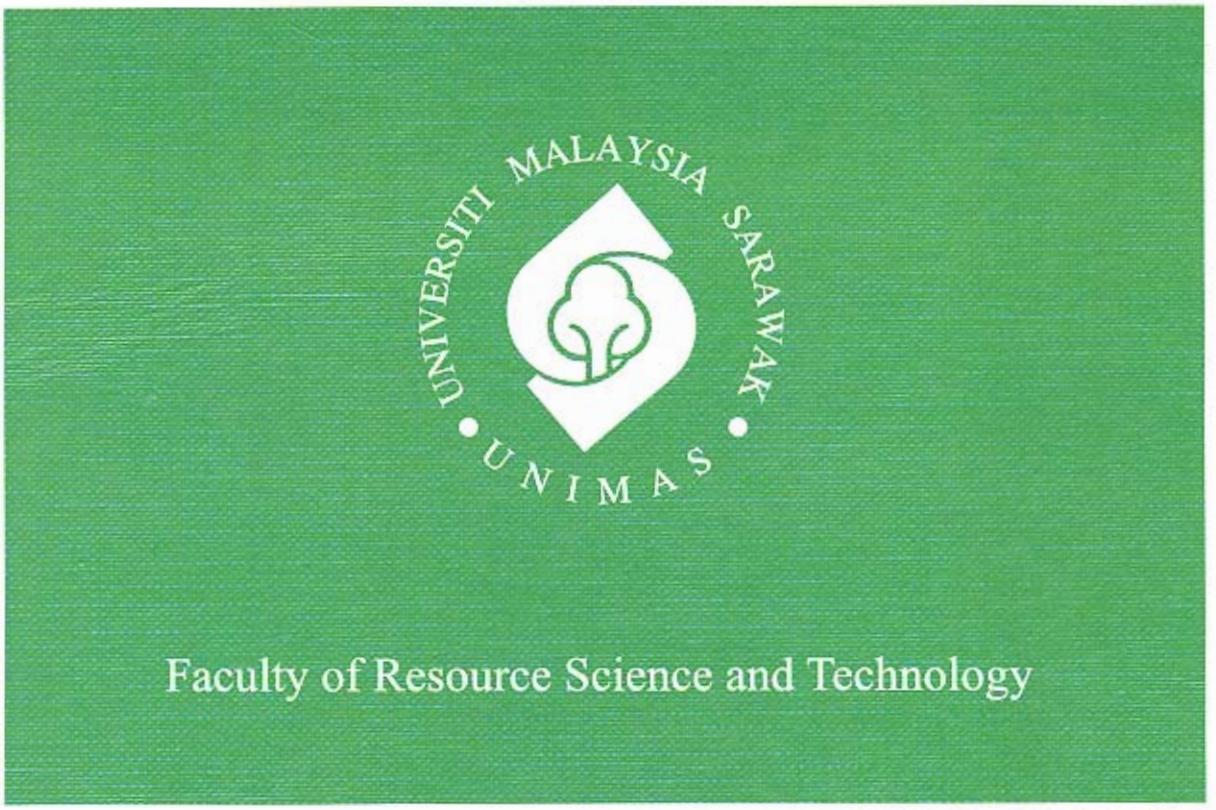

Lactic Acid Separation from Fermentation Broth by Ion Exchange Resin

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Bachelor of Science with Honours
(Resource Biotechnology)
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DECLARATION

I hereby declare that no portion of the work referred in this project has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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(Yong Wei Zhen)

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Department of Molecular Biology

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TABLE OF CONTENT

LIST OF ABBREVIATIONS

LA	Lactic Acid
LAB	Lactic Acid Bacteria
IER	Ion Exchange Resin
SBA	Strong Base Anion
SAC	Strong Acid Cation
LAF	Lactic Acid Fermentation
PLA	Polylactic acid or Polylactide
RPC	Resin Packed Column
L	Liter
Min	Minute(s)
rpm	Revolution per minute
°C	Degree Celsius
M	Molar
H_2SO_4	Sulfuric Acid
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
NH_4OH	Ammonium Hydroxide
ED	Electrodialysis
HPLC	High Performance Liquid Chromatography
RID	Refractive Index Detector

LIST OF TABLES

LIST OF FIGURES

Lactic Acid Separation from Fermentation Broth by Ion Exchange Resin

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ABSTRACT

Lactic acid (LA) promises to be an important commodity chemical in the future for the production of biodegradable polylactic acid. Since the current method for LA separation is expensive and unsustainable, it is vital to use an efficient and sustainable method for downstream processing. Hence, ions exchange resin (IER) method for LA separation was studied with different operating parameters. Some factors like flow rate, resin type, column dimension, and elution agent concentration were systematically investigated to improve the purity, concentration and recovery percentage of LA. In this study, fermentation broth obtained from Biochemistry Laboratory was centrifuged to remove the cells. After that, the sample was loaded into the resin column during service cycle and followed by elution and regeneration cycle. The eluates were then analyzed using HPLC. Amberlite IRA-400 strong base anion exchange resin was mainly applied for the separation of LA from fermentation broth. Results indicated that the decrease of flow rate can improve the recovery percentage of LA. Besides, results showed that Amberlite IRA-400 resin has higher separation efficiency than Amberlite IRA-120 resin. The scale-up of separation process in laboratory size using two different columns exhibits little influence on LA recovery. By increasing the concentration of the elution agent, the maximum concentration of LA at optimum condition was found to be 28.07 g/L using IER method.

Keywords: Lactic acid, separation method, fermentation broth, ion exchange resin

ABSTRAK

Asid laktik (LA) merupakan komoditi kimia yang penting untuk penghasilan terbiodegradasi poli(laktik asid). Memandangkan kaedah semasa bagi pemisahan LA adalah mahal dan kurang mampan, suatu kaedah yang cekap dan manpan adalah penting untuk pemprosesan hiliran. Justeru, kaedah resin pertukaran ion (IER) untuk pemisahan LA telah dikaji dengan menggunakan parameter operasi yang berbeza. Beberapa faktor seperti kadar aliran, jenis resin, dimensi lajur dan agen elusi konsentrasi telah dikaji secara sistematik untuk meningkatkan LA ketulenan, konsentrasi dan peratusan pemulihan. Dalam kajian ini, kaldu penapaian diperoleh dari Makmal Biokimia diprarawat dengan pengemparan bagi membuang sel-sel yang terkandung dalam kaldu. Selepas itu, sampel dimuatkan ke dalam lajur resin semasa kitaran servis dan diikuti oleh elusi serta penjanaan semula. Kemudian, eluat dianalisiskan dengan HPLC. Pertukaran anion Amberlite IRA-400 adalah digunakan terutamanya untuk pemisahan LA dari kaldu penapaian. Keputusan eksperimen menunjukkan pengurangan kadar aliran dapat meningkatkan peratusan pemulihan LA. Selain itu, resin Amberlite IRA-400 mempunyai kecekapan pemisahan yang lebih tinggi daripada resin Amberlite IRA-120. Penskalaan ke atas proses pemisahan bagi saiz makmal hanya mempamerkan sedikit pengaruh ke atas LA pemulihan. Dengan bertambah agen elusi konsentrasi, kepekatan maksimum LA dalam keadaan optimum didapati adalah 28.07 g/L dengan menggunakan kaedah IER.

Kata kunci: Asid laktik, kaedah pemisahan, kaldu penapaian, resin pertukaran ion

1.0 INTRODUCTION

Nowadays, lactic acid (LA) has become a large-volume commodity chemical due to its numerous applications in the food, chemical, pharmaceutical, cosmetic, leather, and textile industries (Kharas *et al.*, 1994; Vickroy, 1985). According to John *et al.* (2008), sodium lactate, a neutralized salt of LA has a mild saline taste and therefore is suitable used for flavor enhancement in meat products. Besides, they also reported that the primary classes of consumer products such as new green solvents (ethyl lactate) and cleaning agents are produced from LA. Recently, LA consumption has increased considerably because of its role as monomer in the production of biodegradable poly-lactic acid (PLA) (Litchfield, 1996).

Lactic acid can be produced by either microbial fermentation or chemical synthesis. According to Wee *et al.* (2006), fermentation process offers an alternative to the limited supply of petrochemical resources and able to reduce environmental pollution due chemical LA production. Moreover, through microbial fermentation, an optically pure LA that is crucial for commercial uses can be produced (Lunt, 1998). Biotechnological processes for the production of LA usually include LA fermentation and product recovery or purification. According to Nolasco-Hipolito *et al.* (2002), the cost of recovery in downstream processing is the main economic factor in any industrial organic acid production. Hence, numerous separation techniques have been studied as separation process takes a large part of the total cost in LA production (John *et al.*, 2008).

Many separation methods such as electrodialysis, membrane filtration, liquid-liquid (solvent) extraction and ion exchange have been studied for LA recovery (Boniardi *et al.*, 1997; Chen and Lee, 1997; Evangelista and Nikolov, 1996; Planas *et al.*, 1999). In electrodialysis units, cells adhered to membranes during the process can lower the

recovery efficiency in the system (Moldes *et al.*, 2003). Besides, relatively high amounts of solvents is required during solvent extraction process can bring toxicity to the environment (John *et al.*, 2008). However, a study by John *et al.* (2008) found out that ion exchange resin (IER) is a practical method from an industry point of view due to its cost-effectiveness, less chemical consumption and less production of waste. Therefore, an economical, efficient, and eco-friendliness separation method like IER is vital to recover LA from fermentation broth.

Ion exchange technique has been used extensively in bio-separation process and several different ion exchangers have been studied on the recovery of LA in the past few years (Tong *et al.*, 2004). However, little systematic study has been done on the effects of different operating conditions on the separation efficiency of LA from fermentation broth using IER method. Hence, the objectives of this study are to investigate the effects of different operating parameters on the LA recovery and finally gain a purified product of LA from fermentation broth with higher purity using single-step IER chromatography.

2.0 LITERATURE REVIEW

2.1 Lactic Acid

Lactic acid (LA) is also known as 2- hydroxypropanoic acid. It is an organic carboxylic acid with a chemical formula of $C_3H_6O_3$ and was first isolated in 1970 by Carl Wilhelm Scheele. It can be found primarily in sour milk products such as yogurt. LA consists of a hydroxyl group adjacent to the carboxyl group and the structure of LA is shown below.

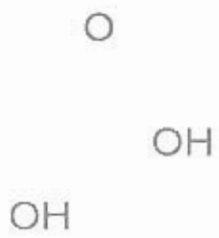


Figure 1: Structure of lactic acid.
Adapted from http://www.creovino.com/archives/39

Lactic acid is a weak monoprotic acid that only partially dissociates in aqueous solution, producing a proton (H^+) and the lactate ion ($C_3H_5O_3^-$). LA is a hygroscopic substance (humectant) that increases skin moisture (Wee *et al.*, 2006). LA exists as two isomers that are L(+)-lactic acid and D(−)-lactic acid. The main difference between these two optical isomers is L-isomer is classified as GRAS (generally recognized as safe) product whereas D-isomer is harmful to human metabolism (Datta *et al.*, 1995). Also, L-LA is an important biological isomer since it involves in the formation of poly-L-lactide (PLA) that contributes to the production of biodegradable plastics (Evangelista and Nikolov, 1996; Kharas *et al.*, 1994; Vickroy, 1985). Hence, consumption of LA will be growing rapidly in next several decades due to its biodegradability characteristics.

There is approximately 90% LA produced worldwide every year are made by LA bacterial fermentation and whereas the rest are by chemical synthesis from petrochemical resources (Hofvendahl and Hahn-Hägerdal, 2000). The hydrolysis of lactonitrile during chemical production always produces racemic mixture DL-LA whereas an optically pure L-isomer or D-isomer can be produced via microbial fermentation by choosing the appropriate strains of bacteria (Wee *et al.*, 2006). By using this biotechnological production of LA, it can provide benefits such as reduce the environmental pollution caused by the chemical synthesis of LA in petrochemical industry.

2.2 Lactic Acid Fermentation (LAF)

In LAF, microorganisms that can be used to produce LA are divided into two groups: bacteria and fungi (Litchfield, 1996). The examples of microorganisms are filamentous fungi such as *Rhizopus* whereas lactic acid bacteria (LAB) are *Lactococcus lactis, Lactobacillus casei, Enterococcus faecalis* and others. For LAB, it can be classified into two groups: homofermentative and heterofermentative.

Hofvendahl and Hahn-Hägerdal (2000) reported that the homofermentative LAB convert glucose almost exclusively into LA whereas the heterofermentative LAB convert glucose into not only LA, but also carbon dioxide as well as ethanol. According to Yun *et al.* (2003), only the homofermentative LAB are suitable for commercial production of LA since homofermentative LAB usually metabolized glucose through glycolysis which results in LA as the major end product. The major fermentation product, LA has been used extensively in the food industry, pharmaceutical, cosmetics, textile, leather industry and also in medical field (Hossain and Maisuria, 2008; Milcent and Carrere, 2001).

2.3 Lactic Acid Separation and Purification Techniques

As environment is a major concern to industrial, a cost-effective and environmental friendly separation method is essential for LA recovery. Therefore, various techniques of separation were studied because the overall cost of the production is dominated by LA separation and recovery cost (Aljundi *et al.*, 2005; Drioli *et al.*, 1996; Han *et al.*, 2000).

To obtain high throughput LA recovery from fermentation broth, various techniques have been developed such as solvent extraction (Planas *et al.*, 1999), electrodialysis (Boniardi *et al.*, 1997), ion exchange resin method (Srivastava *et al.*, 1991), etc. However, the organic solvents used in solvent extraction are generally expensive and can pose potential environmental hazard (Banik *et al.*, 2003). For example, solvents such as aliphatic amines can cause ecological toxicity (Banik *et al.*, 2003) and eventually affect the downstream recovery cost. Thus, the successful development of solvent extraction method requires careful selection of a highly efficient and nontoxic solvent for extraction purpose. Furthermore, this technique is not preferred because it can cause several physical, chemical and biochemical problems on the catalytic activity of cells (Yabanavar and Wang, 1985).

On the other hand, Boniardi *et al.* (1997) stated that electrodialysis (ED) technique is potentially useful in the recovery of LA from fermentation broth. However, the process is complicated and expensive because the wastewater generated after the ED operation has generally high biological oxygen demand (Harrison *et al.*, 2002). Thus, the treatment of resulted wastewater will lead to an increase in LA production cost. Besides that, Yao and Toda (1990) stated that by using ED method, ionic nutrients such as sulfate and

phosphate ions in fermentation broth were electrodialyzed simultaneously with lactate ions, resulting in decrease in the productivity of LA.

According to Tong *et al.* (2001), ion exchange technique is widely used in bio-separations. In the past few years, several different ion exchangers have been investigated for lactic acid recovery from fermentation broth such as PVP resin (Zheng *et al.*, 1996), Amberlite IRA-420 (Antonio *et al.*, 2000), Amberlite IRA-400 (Srivastava *et al.*, 1991), Dowex-50W resin (Chol and Hong, 1999), etc. This technique seems to be beneficial from an industrial point of view due to its cheap maintenance and operational cost, the long life of resins, and this method is relatively environmental friendly compared to other techniques (Ataei and Vasheghani- Farahani, 2008; Srivastava *et al.*, 1991). Therefore, an efficient, simple and economical separation method such as IER is required for the downstream process to recover the lactic acid from fermentation broth.

2.4 Ion Exchange Resin

Ion exchange resin (IER) is an insoluble matrix cross-link porous polymer substance which containing attached functional group. They are polymers that are capable of exchanging particular ions within the polymer with ions in a solution that is passed through them (Zagorodni, 2007). IER can be classified as acid cation exchangers, which exchangeable ions are positively charged, and base anion exchangers which exchangeable ions are negatively charged (Sata, 2004). They can be distinguished through the ionizable (functional) group attached to the hydrocarbon network.

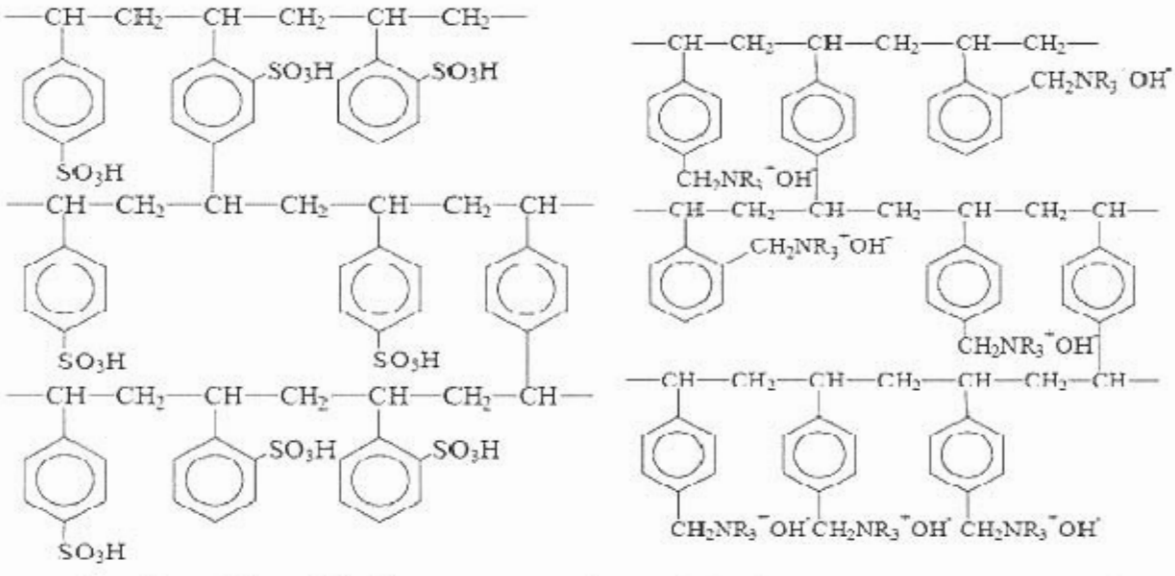
IER can be generally categorized into four types: strong or weak acid cation exchangers and strong or weak base anion exchangers. In SBA resins, they can be further

divided into 2 groups: type 1 and type 2 resins. Chemically, the two types differ in the species of quaternary ammonium exchange sites: type 1 sites contain three methyl groups, whereas in type 2 sites have an ethanol group replaces one of the methyl groups. In the perspectives of the selectivity of resins, its affinity is influenced by the properties of the bead, the ions being exchanged, and the solution in which the ions are present (Pierre and Roberge, 2000). Generally, IER will have greater selectivity for ions with increasing valence or charge and higher affinities are seen for ions with higher atomic number among ions with the same charge.

Table 1: Types of ion exchange resins and its functional group.

Ion Exchange Resin Types	Functional Group
Strong Acidic Cation Exchange Resins (SAC)	Sulphonic acid, $—SO_3^- H^+$
Weak Acidic Cation Exchange Resins (WAC)	Carboxylic Acid, —COOH
Strong Basic Anion Exchange Resins (SBA)	Quaternary ammonium, $—N(CH_3)_3^+OH^-$
Weak Basic Anion Exchange Resins (WBA)	Amines , $—N(CH_3)_2$

In terms of physical structure, IER can be manufactured either in gel (microporous) or macroporous resins. According to Dardel and Arden (2008), gel resins usually have higher operating efficiency and less costly whereas macroporous resins provides better physical stability due to its sponge-like structure that gives more stress relief. In terms of chemical structure, there are two basic types: styrene and acrylic and divinylbenzene (DVB) is a cross-linker used in both types of resins. The styrene-based resins are aromatic hydrocarbons while acrylic resins are straight-chained hydrocarbons.



A strongly acidic sulphonated polystyrene cation exchange resin

A strongly basic quaternary ammonion anion exchange resin

Figure 2: Some examples of ion exchange resin.

Adapted from http://nzic.org.nz/ChemProcesses/water/13D.pdf

2.5 Applications of Ion Exchange Resin (IER) in Separation Process

The two most ion exchange (IE) techniques that commonly applied are batch mode and column mode (Fweja *et al.*, 2010). In addition to that, an expanded bed adsorption (EBA) is also another way to perform an ion exchange separation (Feuser *et al.*, 1998).

According to Zagorodni (2007), batch operations are rarely used in industrial application and the main drawback of batch process is that it cannot separate the ions completely if the resin material has a moderate affinity. Conversely, column technique has been widely used in practical applications. In ion exchange column chromatography, the changes of the composition of eluant passing through the resin bed are mainly depend on the factors such as operating conditions, properties of the ion exchangers, shape or dimension of the column, and composition of the feed solution.

According to Rao (2010), ion exchange chromatography works on the principle of electrostatic attraction between the solute and the charged groupings on the adsorbent (resins). It is a reversible exchange process that involves solvent and the solute ions. Typically, the operating cycle of an ion exchange column system comprises of loading (service) cycle, backwash cycle, regeneration cycle and rinse cycle (George *et al.*, 2010). IER are found in many numerous commercial applications which including the removal of heavy metals from industrial effluents (Nabi *et al.*, 2005), biodiesel purification (Berrios and Skelton, 2008), purification of pharmaceutical active ingredients (Mahore *et al.*, 2010), and separation of proteins and peptides (Khademi and Mostafaie, 2010).

3.0 MATERIALS AND METHODS

3.1 Materials and Samples

Amberlite IRA-400 (Cl^- form) anion exchange resin was purchased from Organo Corporation, Japan and Amberlite-IRA 120 (H^+ form) cation exchange resin was purchased from Aldrich. The other chemicals and fermentation broth mainly composed of lactate ions used in this study were obtained from Biochemistry Laboratory, UNIMAS.

3.2 Preparation and Pretreatment of the Fermentation Broth

The pH of the broth was adjusted to 7.5 by adding 1 M NaOH. The fermentation broth (1 L) was centrifuged at a speed of 10, 000 rpm, at temperature 4°C for 15 minutes. The supernatant was then transferred into a new bottle and autoclaved. The cell-free broth was then filtered using Whatman 0.45 μm cellulose membrane filters before loaded into the service cycle. For the isolated bacterial cells, it can be reused in other fermentation process.

3.3 Preparation of the Ion Exchange Resin

When possible, it is desirable to soak the resins in distilled water or ultrapure water overnight before being placed in the service vessel (column). This is because to make sure that they are fully hydrated. For resin storage, it can be stored under the distilled water.

3.4 Operations of Anion Exchange Resin Using Column Chromatography

3.4.1 Addition of Concentrated Sodium Hydroxide (Regeneration Cycle)

Amberlite IRA-400 (strong base anion exchange resin) was hydroxylated and converted to the hydroxide form. This is because chloride ion has high electronegativity compared to lactate ions. A desired volume of resin (30 ml or 60 ml) was measured with a graduated cylinder and then loaded into a sintered glass column with taper and successively hydroxylated with about 4 bed volumes of 1.5 M NaOH (regenerant). Next, sufficient distilled or ultrapure water was added until the pH of the effluent was the same as that of the wash water (pH 6- 7). The pH was tested with indicator paper (red litmus paper) by taking a drop of effluent to piece of paper on a watch glass.

3.4.2 Service (Loading) Cycle

A desired volume of broth (15 ml or 30 ml) was added into the resin column and the effluent was collected in a conical flask and analyzed with HPLC. Next, the resins column was rinsed with sufficient distilled water until the pH of the effluent was the same as that wash water. This can be tested with indicator paper (red litmus paper) as mentioned above.

3.4.3 Elution Cycle

Next, a desired volume (15 ml or 30 ml) of concentrated 1.0 M HCl (eluant/ elutant/elution agent) was added into the resin column to carry out an elution. The product resulted from elution was collected and analyzed with HPLC. Next, the resin

column was rinsed with sufficient distilled water until the pH of the effluent was the same as the washed water. The pH was tested with blue litmus paper. Next, regeneration process was performed by using concentrated 1.5 M NaOH solution as mentioned above and the resin column was ready to be used for the next service cycle.

3.5 Operations of Cation Exchange Resin Using Column Chromatography

A desired volume of broth (15 ml or 30 ml) was added into the resin column (Amberlite IRA-120) and the effluent was collected in a conical flask and analyzed with HPLC. Next, the resin column was rinsed with sufficient distilled water until the pH of the effluent was the same as that wash water. This can be tested with indicator paper (blue litmus paper) as mentioned above. During regeneration cycle, 4 bed volumes of 1.0 M HCl (regenerant) were used and then subsequently rinsed with distilled water and the resin column was ready to be used for the next service cycle.

3.6 HPLC Analytical Method

Concentration of LA and other compounds in fermentation broth were determined by High Performance Liquid Chromatography (HPLC). HPLC analysis was performed on a Shimadzu (Kyoto, Japan) chromatographic system that equipped with Shimadzu LC-20AT (four pumps) and Shidmadzu-RID-10A refractive index detector. Chromatographic separation was performed on a fermentation monitoring column, Aminex (7.5mm × 150mm). The mobile phase used was ultrapure water with 0.005 M sulphuric acid. The flow rate was set at 0.8 ml/min and the temperature was set at 60 °C. The injection volume was 20 ul.

Figure 3: HPLC analyzer equipped with Shimadzu chromatographic system.

Table 2: Properties of a commercial strong base anion exchanger (Amberlite ® IRA-400 Cl).

Properties	
Type	Strongly basic, Type I
Structure	Gelular (Microporous)
Matrix	Styrene divinylbenzene (DVB) copolymer
Functional group	Quaternary ammonium, $-N(CH_3)_3^+$
Physical form	Insoluble, pale yellow translucent beads
Ionic form	Chloride form
Total exchange capacity	≥ 1.40 meq/ml (Cl^- form)

Table 3: Properties of a commercial strong acid cation exchanger (Amberlite ® IRA-120 H).

Properties	
Type	Strongly acidic
Structure	Gelular (Microporous)
Matrix	Styrene divinylbenzene (DVB) copolymer
Functional group	Sulphonic acid, $-SO_3H^-$
Physical form	Insoluble, amber beads
Ionic form	H^+
Total exchange capacity	≥ 1.80 meq/ml (H^+ form)

4.0 RESULTS AND DISCUSSION

4.1 Pretreatment of Fermentation Broth and Maintenance of Resin

Fermentation broth was first undergone series of pretreatment before utilized in the studies of resin column adsorption and separation. Firstly, the broth was centrifuged to eliminate the cells and other suspended solids present.

Low suspended solids in fermentation broth is essential, especially in a resin packed column (RPC) because suspended solids in the sample could accumulate on the resin bed during the loading (service) cycle. When this particulate matters remain in the resin bed, they will occupy the void areas of the resins, lead to an increasing pressure drop across the bed (Asadi, 2007). This will eventually lead to channeling, clumping and fouling of the resin bed (Skriba *et al.*, 1981).

After centrifugation, the fermentation broth was autoclaved to prevent the further growth of microorganisms. This is because the same fermentation broth was used throughout the ion exchange resin column studies. In this regard, it is possible to reduce the microbial fouling when the resin is left standing in the regenerant during non-operating times. This bactericidal effect can be utilized because the contaminated resin exchangers cannot be properly sanitized through flushing, backflushing or other rinsing processes (Flemming, 2003).

However, protein and dissolved organic matter may still present in the pretreated broth which imparts a yellowish color although the turbidity of broth has been reduced after centrifugation as shown in **Figure 4.** These nutrients can become irreversibly adsorbed within the resin beads, thus will compete for the ion exchange sites and lowering its effective capacity for the lactate ions (Moldes *et al.*, 2003). However, several